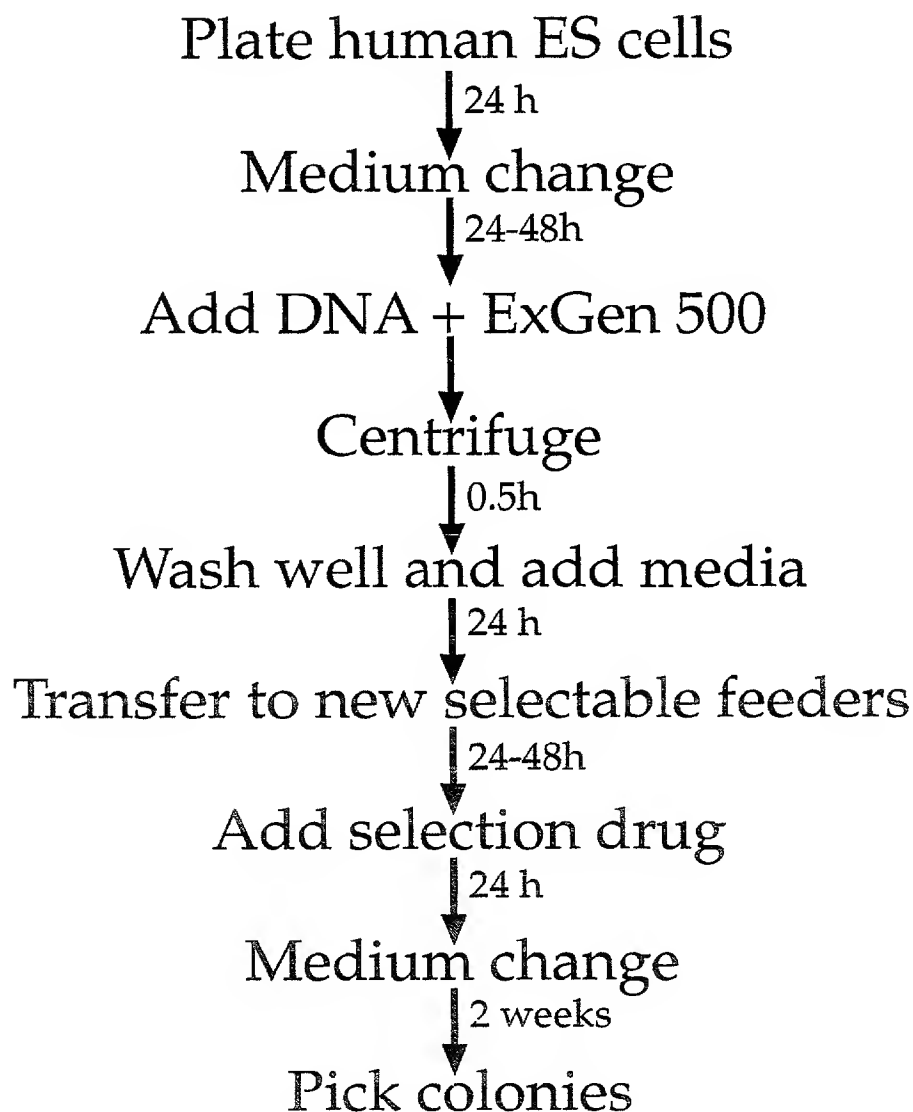


Transfection of human embryonic stem cells



What is claimed is:

1. A method of altering gene expression in a population of human embryonic stem cells; comprising:

introducing a polynucleotide into the population of cells, the
5 polynucleotide containing a gene expression altering sequence so that gene expression in the embryonic stem cells prior to introducing the polynucleotide is measurably different from gene expression after introducing the polynucleotide while retaining the pluripotent character of the cells.

2. A method according to claim 1, wherein the expression altering sequence
10 is an enhancer for modulating gene expression in the population of embryonic stem cells.

3. A method according to claim 1, wherein the expression altering sequence is a gene encoding a protein, the protein not expressed in the population of embryonic stem cells absent the polynucleotide.

4. A method according to claim 3, wherein the protein is selected from a
15 fluorescent protein and an antibiotic resistance protein.

5. A method according to claim 4, wherein the fluorescent protein is selected from green fluorescent protein, lacZ, firefly Rennila protein, luciferase, red cyan protein and yellow cyan protein.

6. A method according to claim 4, wherein the antibiotic resistance protein is
20 selected from hygromycin, neomycin, zeocin and puromycin.

7. A method according to claim 1, wherein the polynucleotide is formulated with a cationic non-lipid polymer transfection reagent for introducing the polynucleotide into the population of cells.

8. A method according to claim 1, wherein the polynucleotide is formulated
25 with a liposomal transfection reagent for introducing the polynucleotide into the population of cells.

9. A method according to claim 1, wherein the polynucleotide is formulated with a cationic lipid reagent for introducing the polynucleotide into the population of cells.

10. A method according to claim 1, wherein the polynucleotide is introduced
30 by electroporation into the population of cells.

11. A method of altering gene expression in a population of human embryonic stem cells; comprising:

introducing into the population of cells by electroporation or in the

presence of a cationic polymer, a DNA sequence corresponding to at least one of an enhancer, a promoter and a gene so as to alter gene expression in the population of embryonic cells in an amount to permit cells containing the DNA sequence to be distinguished from cells absent the DNA sequence.

5 12. A method according to claim 11, wherein the DNA sequence corresponds to a gene and the gene encodes a protein selected from a fluorescent protein, a suicide gene, and an antibiotic resistance protein.

 13. A method according to claim 11, wherein the DNA sequence corresponds to a promoter and the promoter is selected from rex-1, oct-4, oct-6, SSEA-3, SSEA-4,
10 TRA1-60, TR1-81, GCTM-2, alkaline phosphatase, and Hes1 promoters.

 14. A method according to claim 12, wherein the fluorescent protein is selected from green fluorescent protein, lacZ, firefly Rennila protein, luciferase, red cyan protein and yellow cyan protein.

 15. A method according to claim 12, wherein the protein is an antibiotic
15 resistance protein and the antibiotic resistance protein is selected from hygromycin, neomycin, zeocin and puromycin.

 16. A method according to claim 12, wherein the DNA corresponds to a suicide gene and the suicide gene is an inducible apoptic gene or encodes a protein selected from herpes simplex thymidine kinase, inducible Diphtheria toxin, bacterial
20 cytosine deaminase.

 17. A method according to claim 11, wherein DNA sequence causes a knockout of a genomic sequence the genomic sequence selected from beta 2 microglobulin, HLA-1, HLA-2 or an INF receptor gene sequences.

 18. A method for purifying pluripotent embryonic stem cells from a
25 heterogeneous population of cells, comprising:

 (a) introducing into the cells, a DNA encoding a selectable marker under a promoter that is specifically active in undifferentiated cell;

 (b) separating those cells expressing the selectable marker from cells not expressing the marker; and

30 (c) obtaining purified pluripotent cells.

 19. A method according to claim 18, wherein the selectable marker is a fluorescent marker.

 20. A method according to claim 18, wherein the fluorescent marker is selected from green fluorescent protein, lacZ, firefly Rennila protein, luciferase, red cyan

protein and yellow cyan protein.

21. A method according to claim 18, wherein (b) further comprises separating the cells containing the marker from the cells lacking the marker using a fluorescent activated cell sorter or a laser scanning cytometer.

5 22. A method according to claim 18, wherein the selectable marker is an antibiotic resistance marker.

23. A method according to claim 22, wherein step (b) further comprises separating cells by culturing the cells in a selective medium containing antibiotic.

24. A method for treating a human subject for a condition resulting from a
10 deficiency of a selected cell type, comprising:

(a) causing human embryonic stem cells to be transfected *in vitro* with a nucleic acid encoding a marker under a tissue specific promoter;

(b) separating the selected cell type expressing the marker from cells not
expressing the marker, and

15 (c) introducing the selected cell type into the subject so as to treat the condition.

25. A method according to claim 24, wherein the nucleic acid further contains a suicide gene.

26. A method according to claim 25, wherein the suicide gene is an inducible
20 apoptic gene or encodes a protein selected from herpes simplex thymidine kinase, inducible Diphtheria toxin, and bacterial cytosine deaminase.

27. A method according to claim 24, wherein the marker is selected from a fluorescent marker and an antibiotic resistance protein.

28. A method according to claim 27, wherein the fluorescent protein is
25 selected from green fluorescent protein, lacZ, firefly Rennila protein, luciferase, red cyan protein and yellow cyan protein.

29. A method according to claim 27, wherein the antibiotic resistance protein is selected from hygromycin, neomycin, zeocin and puromycin.

30. A method according to claim 24, wherein the cells are transfected by
30 means of a cationic polymer transfection reagent.

31. A method according to claim 24, wherein the nucleic acid does not contain viral genes.

32. A method according to claim 24, wherein the cell type is selected from epidermal cells, dermal cells, muscle cells, cartilage cells, osteoblasts, osteoclasts,

neurons, retinal cells, endodermal cells, hematopoietic cells, cells of the immune system.

33. A method according to claim 24, wherein the cell type is selected from specialized cells from any functionally distinct organ in the human subject.

5 34. A method according to claim 24, wherein introducing the cell type to the subject is achieved by injection.

35. A method according to claim 24, wherein the condition is selected from cancer, an immune disorder, an autoimmune disease, a disease of aging, a degenerative disease including a neurodegenerative disease, and trauma.

10 36. A cell population; comprising a substantially pure population of human embryonic stem cells containing an expression altering sequence of exogenous DNA.

37. A method of producing a clonal pluripotent cell population from a mixture of pluripotent and differentiated cells; comprising:

(a) transfecting the mixture of cells in the presence of a cationic polymer or by electroporation with a DNA encoding a marker protein under a promoter that is selectively active in cells of the inner cell mass of the embryo; and

(b) separating the embryonic stem cells from the differentiated cells according to the presence or absence of an expressed marker so as to produce the clonal pluripotent cell population.

20 38. A method according to claim 37, wherein the promoter selected from rex-1, oct-4, oct-6, SSEA-3, SSEA-4, TRA1-60, TR1-81, GCTM-2, alkaline phosphatase, and Hes1 promoters.

39. A method according to claim 37, wherein the cell population is transfected with a DNA in the presence of a cationic polymer.

25 40. A method according to claim 37, wherein the marker protein is selected from a fluorescent protein and an antibiotic resistance protein.

41. A method according to claim 37, wherein the fluorescent protein is selected from green fluorescent protein, lacZ, firefly Renilla protein, luciferase, red cyan protein and yellow cyan protein.

30 42. A method according to claim 37, wherein the antibiotic resistance protein is selected from hygromycin, neomycin, zeocin and puromycin.

43. A method of regulating cell viability of a population of cells in a subject, wherein the population of cells are derived from a human embryonic stem cell culture which has undergone directed differentiation, comprising:

(a) introducing the population of cells into the subject, the population of cells containing an exogenous DNA encoding a suicide gene, wherein the population of cells are selected from the group consisting of: undifferentiated cells, partially differentiated or wholly differentiated cells; and

(b) treating the subject with an agent for activating a sequence of events leading to suicide in the cells in the subject in response to an adverse event associated with the introduced cells.

44. A method according to claim 43, wherein the occurrence of adverse events is a hyperproliferation of the introduced cells.

45. A method for screening an agent to determine an effect on differentiation of pluripotent cells *in vitro*, comprising:

(a) adding the agent to an *in vitro* cell culture of a population of genetically engineered human embryonic stem cells expressing a detectable marker under a cell specific promoter; and

(b) providing the conditions for the embryonic stem cells to differentiate; and

(c) determining the effect of the agent on differentiation of pluripotent cells.

46. A method according to claim 45, wherein the detectable marker is a fluorescent marker.

47. A method according to claim 46, wherein the fluorescent marker is enhanced green fluorescent protein.

48. A reagent cell population for supplying material for transplantation consisting essentially of pluripotent human embryonic stem cells modified by foreign genetic material which is DNA not normally present in embryonic stem cells; which occurs in embryonic stem cells but is not expressed in them at levels which are biologically significant; DNA which occurs in embryonic stem cells and has been modified so that it is only expressed by selected derivative cells; or any DNA that can be modified to be expressed by embryonic cells, derivative cells alone or in any combination thereof.

49. A reagent cell population according to claim 48, in which the foreign genetic material comprises genetic material encoding at least one selectable marker.

50. A reagent cell population according to claim 49, in which at least one selectable marker is a dominant selectable marker.

51. A reagent cell population according to claim 50, in which the dominant selectable marker is a gene encoding antibiotic resistance.

52. A reagent cell population according to claim 49, in which the dominant selectable marker is a gene encoding a suicide protein.

5 53. A reagent cell population according to claim 50, in which the dominant selectable marker is a gene encoding a fluorescent protein or an antibiotic resistant protein.

54. A reagent cell population according to claim 52, in which the gene is *hsv-tk* and the suicide protein is thymidine kinase or the suicide gene is an inducible apoptic gene or encodes a protein selected from inducible Diphtheria toxin, bacterial cytosine
10 deaminase

55. A reagent cell population according to claim 53, wherein the gene encodes a fluorescent protein selected from green fluorescent protein, lacZ, firefly Rennila protein, luciferase, red cyan protein and yellow cyan protein.

15 56. A method according to claim 53, wherein the antibiotic resistance protein is selected from hygromycin, neomycin, zeocin and puromycin

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